



An Application of the Kipling Method to DNA Validation in the 21st Century

Thinking Ahead

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Planning for the next validation

- Use the same samples you have tested for your old STR multiplex for the next STR multiplex.
- Positive controls, current employees, and NIST SRM components are popular examples, but have limitations.

Using Analysts as Samples...

Genetic Information Nondiscrimination Act of 2008



Long title An act to prohibit discrimination on the basis of genetic information with respect to health insurance and employment.

Acronyms (colloquial) GINA

Enacted by the 110th United States Congress

Effective May 21, 2008

Planning for the next validation

- Consider – buying a large supply of blood (anonymously collected) from a blood bank.
- Characterize as much as you can (nice summer intern project).
- Why?

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Analysis and interpretation of mixed profiles generated by 34 cycle SGM Plus[®] amplification

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Table 2

The final distribution of major and consensus profiles across the template input and ratio range after completion of the RO requested rework.

Ratio	Total Input	Major/minor	Major/consensus	Consensus only	Unduplicated
5:1	1 ng	12		3	1
	500 pg	10 ^a		6	
	250 pg	11 ^a	1	4	
	100 pg	3 ^a	4	9	
	50 pg	2		14	
	Total	38	5	36	1
2:1	1 ng	1		14	1
	500 pg	1 ^a		14	1
	250 pg	1 ^a		15	
	100 pg		1	14	1
	50 pg		1 ^a	15	
	Total	3	2	72	3
1:1	1 ng			8	
	500 pg			8	
	250 pg			8	
	100 pg			8	
	50 pg		1 ^a	7	
	Total	0	1	39	0

One individual had a possible mutation in the Quantifiler primer binding region that underestimated the relative ratios in the study...

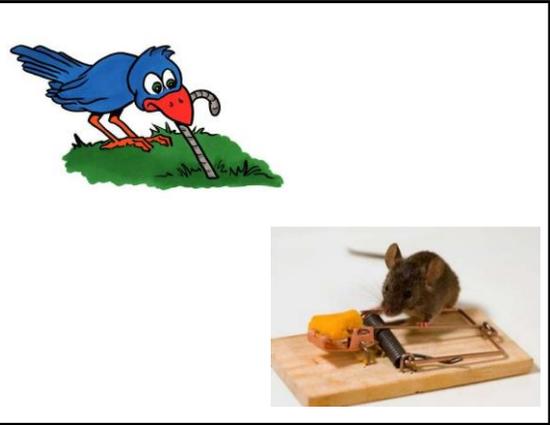
^a One observation in each of these major profile categories was due to a single individual with a possible mutation affecting the accuracy of the Quantifiler[®] concentration estimate which may have caused the input of this individual to be underestimated by half. This would tend to double its relative representation at each mixture ratio as well as the true amount of template available in the PCR. In the other rows the affected mixture was scored as a consensus.

Planning for the next validation

- Don't be an island – consider joining with other labs to plan validation studies. Other labs may have ideas that can streamline your validation.
- Share resources, cost, time. Perhaps you may find a "Steven Myers" type who saves your lab hours with a neat Excel spreadsheet.
- Inter-laboratory challenges to monitor progress.

No matter what you are validating now...

- This is NOT your last validation!
- Should you be an early adopter?
- Should you go kicking and screaming?



Here now...

The screenshot shows a web-based interface for 'LabRetriever'. The main heading is 'Evaluation of Likelihood Ratios'. Below this, there are several menu items: 'Profile', 'Reference Profiles', and 'Frequencies'. A prominent graphic in the center features a DNA double helix with the text 'STRmix' overlaid. In the bottom left corner, there is a logo for 'CYBERGENESIS TrueAllele®'.

Coming very soon...

Two pieces of forensic DNA analysis equipment are shown. On the left is the 'NetBio ANDE' (Automated Next-Generation DNA Sequencer), which is a large, light-colored machine with a control panel. On the right is the 'IntegenX RapidHIT 200', a smaller, white machine with a touchscreen interface. A person's hand is visible interacting with the RapidHIT 200.

Coming later...

Two sequencing platforms are shown. On the left is the 'Ion Torrent' sequencer, characterized by its blue and white color scheme and multiple sample wells. On the right is the 'MiSeq' sequencer, a compact white and black machine with a large touchscreen display.

NGS Thoughts

- Large Multiplexes – SNPs, aSTRs, mtDNA, Y-STRs, Y-SNPs, X-STRs... If data is generated, it must be analyzed.
- Need for allele frequency databases
- Data storage!

Final thoughts

- Proper validation is hard work. It takes a great deal of time and effort to conduct the studies, write the new SOPs, train everyone, and monitor the progress.
- It really shouldn't be "easy" as it's your job to find the limits of the system.

The second stanza of *The Elephant's Child*

- I KEEP six honest serving-men
(They taught me all I knew);
Their names are What and Why and When
And How and Where and Who.
- I send them over land and sea,
I send them east and west;
But after they have worked for me,
I give them all a rest.

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